LWOFFIA CILIFERA GEN. NOV., SP. NOV., A CILIATED MEMBER OF THE FAMILY SPHENOPHRYIDAE (HOLOTRICHA: THIGMOTRICHA)

EUGENE N. KOZLOFF

Lewis and Clark College, Portland, Oregon

Up to the present time, three genera have been referred to the family Sphenophryidae. Two of these, *Pelecyophrya* and *Gargarius,* are monotypic. The third genus, *Sphenophrya,* includes several species, the most extensively studied one being *S. dosiniae.* An excellent summary of our knowledge of this remarkable group of ciliates has been prepared by Chatton and Lwoff (1949, 1950). Since the publication of their comprehensive monograph, one new species has been added to the genus *Sphenophrya* by Polianskiĭ (1951). A contribution of Raabe (1949), in which the development of *S. dosiniae* is compared with that of *S. sphaerii,* is apparently the only other publication dealing with this group which was not issued in time for consideration by Chatton and Lwoff.

The known genera of sphenophryids are parasitic on the gills of lamellibranch mollusks. As adults they are sessile and, with occasional exceptions in *Pelecyophrya tapetis,* are not ciliated. However, an infraciliature, disposed in two systems of a few rows each, persists in adult sphenophryids. In *P. tapetis* and the several species of *Sphenophrya,* the adults produce temporarily ciliated embryos which resemble in a general way some of the ciliates of the family Ancistrocomidae. In *Gargarius gargarius,* the embryo is also ciliated, but it is similar to the adult in all other respects by the time division has been completed. The embryonic ciliature of sphenophryids originates from the basal granules of those portions of the infraciliary rows which are conferred upon the embryo during division.

On the branchial filaments of *Brachidontes* (*Mytilus*) *recurvus* (Rafinesque) I have found a ciliate which is referable to the Sphenophryidae, but which differs from the other genera in having cilia throughout its life history. The morphology of this ciliate is most nearly like that of *Pelecyophrya,* but the process of division gives rise to two fully differentiated individuals, as is the case in *Gargarius.* The new genus is named for Dr. André Lwoff, of the Institut Pasteur, Paris, in recognition of his contributions to protozoology.

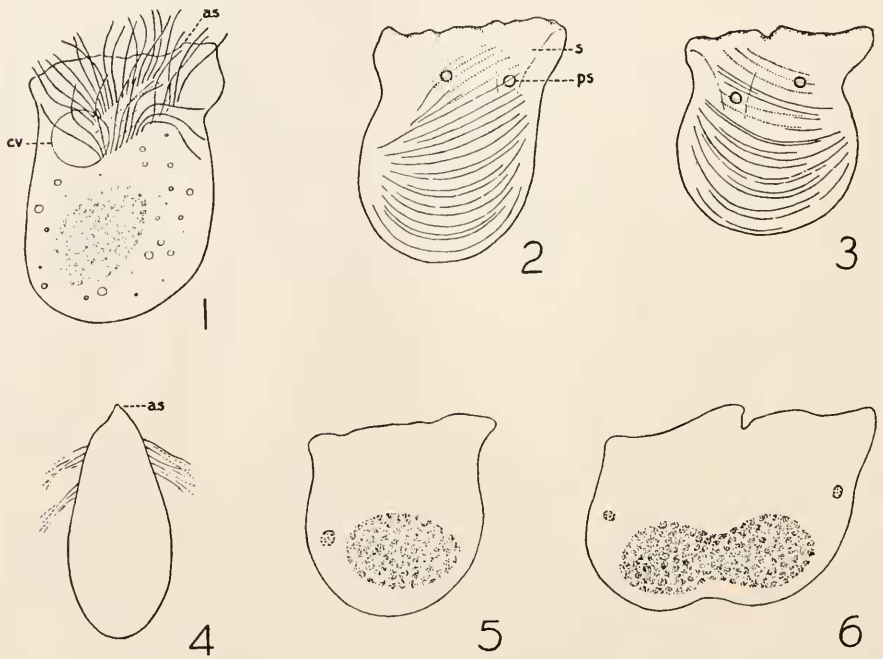
This study was carried out during the tenure of a Fellowship from the John Simon Guggenheim Memorial Foundation. It was begun while I was in residence at the Conservation Reserve of the University of Florida, and completed in the Department of Zoology, University of North Carolina.

Materials and Methods

Lwoffia cilifera was found in only one of ten specimens of *Mytilus recurvus* collected in a brackish lagoon confluent with the Caloosahatchee River approxi-

mately 6 miles southwest of Fort Myers, Florida. In the infected mussel, the ciliate occurred in enormous numbers. This permitted an extensive study of living material and the fixation of smears with hundreds of individuals.

For observation of living ciliates, small pieces of gill tissue were mounted in the fluid drained from the mantle cavity. Smears on coverglasses were fixed in Hollande's fluid with 3 per cent of acetic acid. These were stained with iron hematoxylin or the Feulgen nucleal reaction, or impregnated with activated silver albumose (Protargol) according to the method of Bodian. Impregnation with the sample of Protargol available to me was not satisfactory unless the smears were bleached, after fixation and washing, by Mallory's method, which consists of placing them in 0.5 per cent potassium permanganate for 5 minutes, washing them well, and then treating them with 5 per cent oxalic acid for 5 minutes.



Explanation of Figures 1–6

Lwoffia cilifera. All figures, except Figure 1, have been prepared with the aid of a camera lucida and are based on specimens fixed in Hollande's fluid and impregnated with Protargol (P) or stained by the Feulgen nucleal reaction (F); × 1640.

as, attachment surface; cv, contractile vacuole; ps, primordium of sucker; s, sucker.

Figure 1. From right side; after sketches from life.

Figure 2. From right side, showing rows of basal granules, pellicular fibril-like structures, sucker, and primordia of suckers; nuclei omitted; P.

Figure 3. From left side; P.

Figure 4. Optical section, posterior to middle; P.

Figure 5. Macronucleus and micronucleus; F.

Figure 6. Late stage of division; F.

Lwoffia cilifera gen. nov., sp. nov.

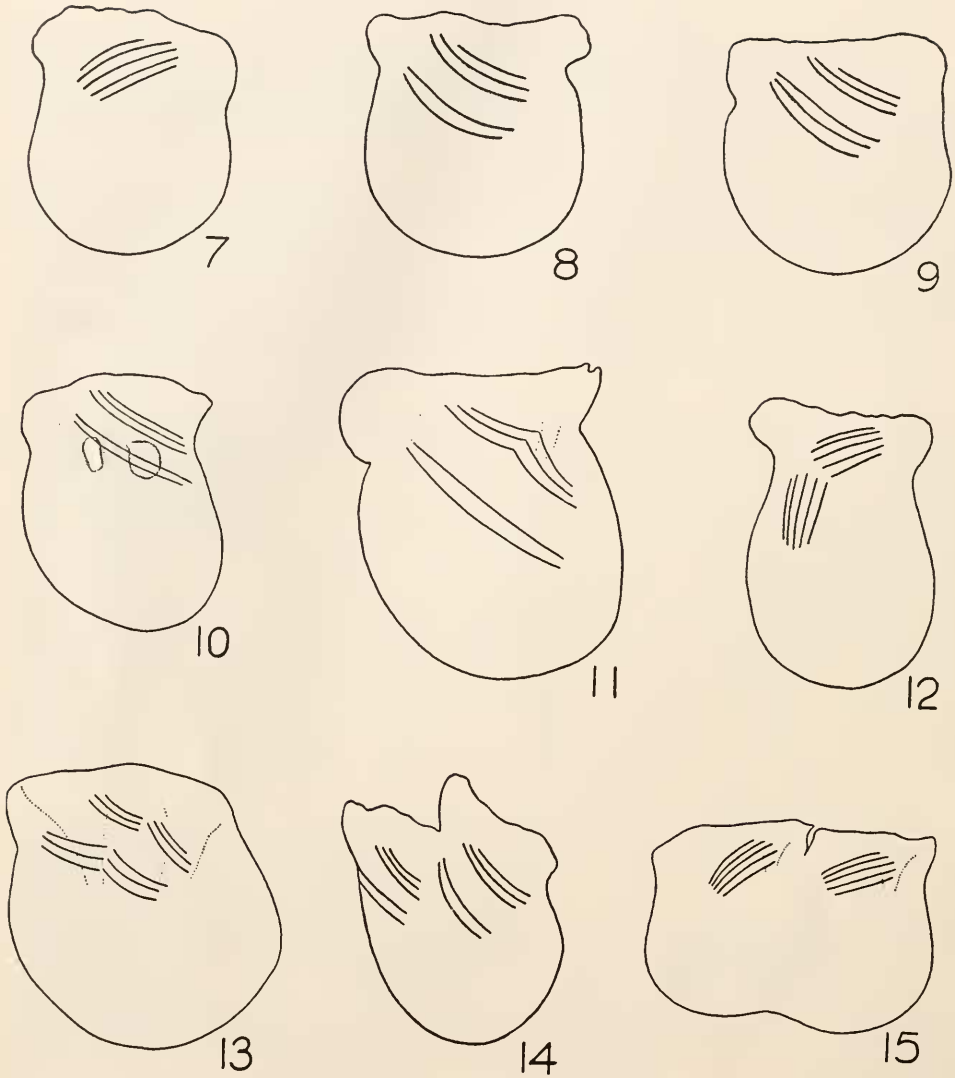
The body is compressed, having a thickness equal to about one-half the breadth of the ciliated surfaces (Figs. 1–4). The wide variation in the shape of the ciliates observed in fresh preparations as well as in fixed smears is due primarily to the plasticity of the ridge-like and somewhat corrugated attachment surface, which is in contact with the branchial tissue of the host.

The distance from the middle of the attachment surface to the opposite pole of the ciliate is usually approximately equal to, or slightly greater than, the greatest breadth below the attachment surface. Twenty living individuals in which these dimensions were measured ranged in size from about 16 μ by 14 μ to about 29 μ by 25 μ. However, as will be explained presently, these dimensions are not comparable to length and width of more conventional ciliates.

The orientation of *L. cilifera* may be defined satisfactorily only with reference to other thigmotrichs. Chatton and Lwoff, after considering the organization of a series of genera of Ancistrocomidae in relation to that of stomatous thigmotrichs such as *Ancistrum*, concluded that the ciliated surface of the more highly evolved ancistrocomids (*i.e.*, those in which the ciliature is reduced to a relatively few rows) is dorsal. In defining the orientation of the three genera of sphenophryids known to them, they regarded the rows of infraciliature in these forms to be homologous with the dorsal ciliary rows of ancistrocomids. In *Pelecyophrya tapetis*, therefore, the rows of infraciliature on either side of the body are collectively considered to correspond to the single system of dorsal ciliature in ancistrocomids. In the ciliated embryo of *P. tapetis*, before it becomes detached, the two ciliary fields destined to lie on opposite sides of the body in the adult converge rather closely upon one another toward the posterior end. The sucker occupies much of that part of the ciliate which is in contact with the gill tissue of its host, and is considered to be antero-ventral in position.

In species of *Sphenophrya*, in which the two systems of infraciliature evidently homologous with those of *Pelecyophrya* lie on the same side of the elongated body, the problem of orientation is more complicated. However, the ciliated embryo of *Sphenophrya* is much like that of *Pelecyophrya*, and during the changes involved in the development of the elongated adult form, the rows of infraciliature disposed on the right side of the embryo eventually come to lie on the same side as the rows which are on the left side of the embryo. The attachment surface was taken by Chatton and Lwoff to be dorsal, since it represents a differentiation and expansion of that portion of the embryo which separates the right and left ciliary systems.

The attachment surface of *Lwoffia* is more extensive than that of *Pelecyophrya*, and is disposed on those portions of the body which correspond to the anterior end and anterior part of the dorsal surface in *Pelecyophrya*. The sucker of *Lwoffia* resembles that of *Pelecyophrya* in having a broad funnel-shaped outline when viewed from the right or left side of the body (Figs. 2, 3). The margins of the sucker may be discerned in some specimens stained with iron hematoxylin, and individuals impregnated with Protargol show them quite regularly. The sucker becomes broader antero-dorsally, and its anterior margin may often be traced as far as the attachment surface. I have been unable to determine how much of the attachment surface is occupied by the origin of the sucker, and I have



EXPLANATION OF FIGURES 7–15

Lwoffia cilifera. All figures have been prepared with the aid of a camera lucida and are based on specimens fixed in Hollande's fluid and impregnated with Protargol; × 1640. The rows of cilia are rendered diagrammatically as continuous lines.

FIGURE 7. From right side.

FIGURES 8, 9. From left side.

FIGURE 10. Enlargement of primordia of suckers.

FIGURE 11. Elongation and early stages of division of ciliary rows.

FIGURES 12–14. Later stages in separation of ciliary systems of prospective daughter ciliates.

FIGURE 15. Attachment surfaces of prospective daughter ciliates distinct.

not observed an actual orifice. The sucker is evidently a protoplasmic channel. Although the cytoplasm of *L. cilifera* contains abundant lipoid granules, stainable with Sudan III in fresh preparations, and smaller, relatively non-refractile granules, cell fragments from host tissue cannot be identified. It appears probable that nutrition of the ciliate involves absorption of relatively fluid materials.

In impregnated specimens there is almost invariably observed within the sucker a small sphere whose periphery is argyrophilic (Figs. 2, 3). Another sphere of this type is characteristically located posterior to the region occupied by the sucker. These two spheres are primordia of new suckers formed during division of the cilate, and may therefore be regarded as homologues of the sucker primordia observed by Chatton and Lwoff in about 30 per cent of the individuals of *P. tapetis* stained with iron hematoxylin. Apparently, however, neither of the two primordia in *P. tapetis* lies within the sucker of the parent ciliate.

The cilia are approximately 8 μ or 9 μ long. After being detached from the gill tissue of its host, *L. cilifera* is capable of swimming actively, although its movements are largely erratic rotations.

In proportion to the size of the body, the ciliary rows are much shorter than the rows of infraciliature in *P. tapetis*. In the latter species, some of the rows extend almost the full length of the ciliate. On the right side of the body of *L. cilifera* there are five rows arranged in one group (Figs. 1, 2, 7). On the left side of the body, there are two groups of rows (Figs. 3, 8, 9), as in *Pelecyophrya*. The group which is nearer to the attachment surface consists of three rows in all suitably oriented impregnated specimens which I have studied. The group which is farther from the attachment surface consists most frequently of two rows, but individuals with three rows in this group are common. In two specimens, out of hundreds I studied, there were four rows in this group, and in one specimen there was a single row.

In Protargol preparations, a number of delicate fibril-like structures, which appear to be pellicular, are observed on the right and left sides of the body, in both the ciliated and unciliated portions (Figs. 2, 3). Many of these, at least, traverse the entire broad surface, and at either end there is a convergence of the fibril-like structures from the right and left sides.[1]

The large macronucleus is located in the postero-ventral half of the body. It is elongated, and is usually about one and a half to two times as long as it is wide. In life, the macronucleus is coarsely granular (Fig. 1), with some of the granules being more or less rod-shaped. After fixation it is stained so heavily by the Feulgen nucleal reaction and by iron hematoxylin that its granular character is often obscured. The micronucleus generally lies close to the macronucleus. In the interphase it is typically spherical, with the chromatin dispersed in rather fine granules (Fig. 5). The contractile vacuole is located in the posterior part of the dorsal half of the body.

Stages of division are abundant in my stained and impregnated preparations of *L. cilifera*. In impregnated specimens, in which nuclear changes cannot be followed satisfactorily, the earlier stages of fission are evidenced by the division of each row of cilia into two segments. The two daughter segments are generally

[1] Comparable configurations are prominent in my Protargol preparations of *Gargarius gargarius* and *Sphenophrya dosiniae*.

approximately equal (Figs. 12–14), and it appears that the parent row elongates considerably before the break in the continuity of the row occurs (Fig. 11). As the furrow of cytoplasmic cleavage becomes more pronounced, the systems of ciliary rows formed by division of the parent system on either side of the body become more widely separated, and the attachment surfaces of the two prospective daughter ciliates become quite distinct (Figs. 14, 15).

During the early stages of division, the primordia of the new suckers to be conferred upon the daughter ciliates enlarge. Evidently this enlargement may be initiated before there is a break in the continuity of any of the ciliary rows (Fig. 10). Since one of the primordia lies within the sucker of the parent ciliate, the new sucker derived from this primordium essentially replaces the old sucker in the same position. As the development of new suckers progresses, the argyrophilic material of the primordia loses prominence.

Division of the micronucleus apparently begins after the primordia have begun to enlarge, and is completed before the attachment surfaces of the prospective daughter ciliates may be recognized as separate. Macronuclear division coincides with later stages of cytoplasmic division (Fig. 6).

Lwoffia gen. nov.

Diagnosis: Sphenophryid ciliates with cilia persisting throughout the life history. The body is laterally compressed, with an extensive ridge-like attachment surface which occupies the anterior end and anterior part of the dorsal surface of the body. The ciliature of the right side is composed of one group of rows; the ciliature of the left side is arranged in two groups of rows. The sucker is funnel-shaped in outline as viewed from the side, and is directed antero-dorsally; it is considered to be a protoplasmic channel. Primordia of new suckers to be formed during division are evident in the interphase, and one of the primordia lies within the sucker of the parent ciliate. Genotype: *Lwoffia cilifera.*

Lwoffia cilifera sp. nov.

Diagnosis: Size approximately 16 μ by 14 μ to 29 μ by 25 μ. The ciliary system of the right side of the body is composed of 5 rows. On the left side of the body, the ciliary rows in the group nearer the attachment surface are three in number, and the rows in the group farther from the attachment surface are commonly two or three in number, rarely one or four. On the branchial filaments of *Brachidontes recurvus* (Rafinesque), near Fort Myers, Florida. Syntype slides are in the collection of the author.

Summary

To the existing genera of sphenophryid ciliates (*Sphenophrya, Pelecyophrya,* and *Gargarius*) described by Chatton and Lwoff, a new genus, *Lwoffia,* is added. The genotype, *L. cilifera,* was found on the gills of *Brachidontes recurvus* near Fort Myers, Florida. Functional cilia persist throughout the life history of *L. cilifera,* and are disposed in two systems on either side of the body, in much the same way as the rows of infraciliature in *Pelecyophrya tapetis.* The attachment surface is proportionately more extensive than that of *P. tapetis,* and in form is much like that of species of *Sphenophrya.* Division produces equal and fully-

differentiated ciliates, rather than an ancistrocomid-like embryo from the parent ciliate, and in this respect is similar to division in *Gargarius gargarius*.

LITERATURE CITED

CHATTON, É., AND A. LWOFF, 1949. Recherches sur les ciliés Thigmotriches. I. *Arch. Zool. exp. gén.*, **86**: 169–253.

CHATTON, É., AND A. LWOFF, 1950. Recherches sur les ciliés Thigmotriches. II. *Arch. Zool. exp. gén.*, **86**: 393–485.

POLIANSKIĬ, IU., 1951. O nekotorykh parazitichеskikh infuzoriiakh iz morskikh molliuskov i goloturii. *Parazitologicheskiĭ sbornik zoologicheskogo instituta Akademiia Nauk S.S.S.R.*, **13**: 355–370.

RAABE, Z., 1949. Recherches sur les ciliés Thigmotriches (Thigmotricha Ch. Lw.). III. Développement non-parallèle de deux espèces du genre *Sphenophrya* Ch. Lw. *Ann. Univ. Mariae Curie-Sklodowska Lublin*, Sect. C, **4**: 119–135.